

**CONTRIBUTIONS  
IN  
MARINE SCIENCES**

**A Special Collection of Papers to Felicitate  
Dr. S.Z. Qasim on his Sixtieth Birthday**

## PERSPECTIVES IN PEARL CULTURE

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### INTRODUCTION

The art of pearl culture, as initially developed by late Kokichi Mikimoto in 1893 and subsequently improved upon by the Japanese scientists and farmers, is in the process of undergoing changes with scientific inputs in new areas. While the traditional pearl culture industry of Japan is looking for improvement in the quality of cultured pearls, several new entrants are coming into the industry. In some areas, there is also interest in reviving the natural populations and improving their pearl producing potential. An industry which had been confined to Japan and a few countries in the Indo-Australian Archipelago under Japanese management is becoming more and more open. Under these changing circumstances, many a discipline of science is getting involved in improving the technology of pearl culture. This paper attempts to bring into focus these science and technology frontiers.

India is one of the few countries which has developed the pearl culture technology indigenously. The work of Alagarswami and Qasim (1973) and Alagarswami (1974a) laid the foundation for pearl culture in India, upon which a research system has been built up and an industry for commercial production has been established. The paper also discusses the recent developments in pearl culture research and development in the country.

### TECHNOLOGY OF CULTURED PEARL PRODUCTION

#### *Origin of technology*

Natural pearl is one of the few gems which came to be associated with human civilization as early as 3500 B.C. The art of pearl culture in its primitive form developed only in the 12th century A.D. in China. Large quantities of 20cm long mussels were collected from Lake Tahu in Kiangsu in Central China. Small outlines of Buddha made of tin, ivory etc. were placed between the inner aspect of shell and the mantle of the mussel. After a year the nacre-coated pearl Buddhas were sawed off the shell and sold in the temple markets<sup>1</sup>. This basic

technique continued in China till recently. The half-pearls or *mabe* are produced in the pearl oyster even today using the same principle.

It took another six centuries to move on to the next phase of pearl culture and the stage shifted to Japan. In the intervening period Linnaeus had engaged himself in an inconclusive experiment to produce pearls in the European mussel in the 17th century. Kokichi Mikimoto of Japan, adopting the Chinese technique, produced half-pearls in the pearl oyster *Pinctada martensii* in 1893. In 1907, Nishikawa propounded the pearl-sac theory stating that a pearl is formed when the pearl-secreting cells of the mantle migrate into the body of the oyster under the stimulus of a foreign body and form a pearl-sac by division which covers the nucleus with nacre. In 1913, Tokichi Nishikawa succeeded in the experimental production of spherical cultured pearls<sup>3</sup>. The pearl culture industry developed from this point by the efforts of both the farmers and the scientists of Japan with marginal contribution from other parts of the world in its more recent phase.

#### *Developments in implantation techniques*

Originally, the shell-bead nucleus was fully wrapped with the mantle tissue and implanted into the visceral mass of the pearl oyster. Later it was found that a small piece of mantle would serve the same purpose. The size of the mantle piece (or graft tissue) was progressively reduced and standard sizes of pieces were evolved for the different sizes of the nucleus. So also the thickness of the epithelium was considered important in determining the quality of the pearl. If it is thin (2 - 10 m), the surface of the pearl would be good. If it is more than 20  $\mu$  m thick, the pearl would be dull and badly coloured<sup>26</sup>. On implantation, the inner epithelium and connective tissue of the mantle piece would disintegrate and get absorbed in the surrounding tissue, leaving the outer epithelial cells to proliferate and cover the nucleus fully, forming the pearl-sac. Generally, the graft tissue is inserted first in position after which the nucleus is implanted. But some do practise the reverse process depending on convenience. For a long time water soluble eosin was used in maintaining the mantle pieces from time of preparation to time of insertion which is normally less than 10 minutes<sup>3</sup>. Eosin has a sterilising effect and also enables visual observation of the passage of the piece during insertion in many cases. Now mercurochrome or other antibiotic solution is used for the purpose<sup>17</sup>.

#### *Conditioning*

Pre-conditioning of pearl oyster for surgery is an essential process in pearl culture. It is aimed at discharge of gametes

and lowering the metabolism of the pearl oyster<sup>3</sup>. The visceral mass is largely occupied by the gonad during the active reproductive phase. Gonad takes the major nucleus load. Hence it is necessary to discharge the gametes for getting the required space for the nuclei to be implanted and also to avoid oozing of gametes through the surgical incision. Lowering of metabolism is done to reduce the reaction of the oyster during surgery, particularly the reaction of adductor and retractor muscles. Thermal stratification in the temperate waters enables achieving the above two processes through maintenance of oysters for short durations in different temperature regimes at different depths. At the higher surface temperatures the oysters spawn naturally. Crowding and suspending in low productive areas for starving the oysters are common practices. In the tropics where such thermal stratification is not present in the inshore waters, this is achieved through narcotisation using menthol crystals. Discharge of gametes of mature oysters has become possible with the development of physical or chemical stimulation techniques<sup>8, 24</sup>.

#### *Techniques for different species*

Pearl production techniques differ from species to species and also on the types of cultured pearls aimed for production. The most common one employed on *Pinctada fucata* (Gould) is for production of free, spherical pearls of diameter range about 2 - 10 mm. This species being the smallest (maximum about 8 cm dorsoventrally) among those employed in pearl culture, it is not suitable for production of half-pearls. Hence all the pearls are grown inside the gonad. About 1-5 pearls can be produced in a single oyster depending on its size<sup>5, 23</sup>.

The largest among the pearl oysters is the goldlip *Pinctada maxima* which produces the South Sea pearls of size upto 20mm. Both spherical pearls and half-pearls are produced in this species. The latter are from nuclei glued to the inner aspects of both the right and the left valves. These oysters are used for a second or even a third crop of pearls because of their size and longevity<sup>16</sup>.

The blacklip pearl oyster *Pinctada margaritifera* is emerging as a species of considerable importance in pearl culture because of the fine, free, black pearls of 10-16mm produced by them<sup>38</sup>. The species was considered more difficult to obtain, to raise, and to use for culturing purposes<sup>26</sup>. However, in the recent years, techniques are continuously being upgraded on implantation and growth control of oysters and pearls in French Polynesia<sup>11</sup>.

The two other marine species *Pteria penguin* and *Haliotis* spp. are used for production of half pearls only and, in terms of quantity, are not as significant as the *Pinctada* spp.

Production of freshwater pearls involves techniques different from those employed on the marine species. *Hyriopsis schlegelii* is the common freshwater mussel used in pearl culture in Japan since 1935. Other species of mussels are now being used in China and U.S.A.<sup>38</sup> Bangladesh is experimenting upon *Lamellidens marginalis* and *Parreysia corrugata*<sup>2</sup>. The species list of freshwater mussels that can be used in pearl culture is expanding with experimental work in different parts of the world and an U.S.A. farm is expected to start marketing large, round pearls from up to 24 species of nucleated mussels in 1987<sup>38</sup>. The traditional technique for the freshwater species has been to produce numerous, small, irregular non-nucleated pearls in the mantle of the mussel by inserting only mantle pieces. In the more recent years it has been technologically possible to produce nucleated, free, spherical cultured pearls in the mussels at least on a limited scale and better techniques are under development<sup>17, 38</sup>

## TECHNOLOGY OF FARMING

Pearl oysters are sedentary organisms and, in the early stages of pearl culture in Japan, *Pinctada fucata* were collected from their natural beds and strewn in demarcated shallower waters in the Ago Bay. Subsequently, to have better control and to avoid predatory problems, off-bottom culture technique was adopted<sup>12</sup>. Oysters were held in bamboo baskets and suspended from rafts. Concurrently the pearl string method was developed to tend them individually facilitating the oysters to have more water space around them and thereby more food. Later, the pearl net was devised to hold 60 or more oysters in each, combining the basket and pearl string techniques, in which more oysters can be held in an unit and, at the same time, the oyster can be monitored individually<sup>3</sup>. The pearl rush of late 1960s appears to have led to a compromise on individual tending, and now multilayered collapsible nets with capacities of more than 300 oysters each, along with pearl nets and baskets, are reported common in pearl culture farms of Japan.

On-bottom culture in holding systems appears to be favoured in the case of *Pinctada margaritifera* in the lagoon of Marutea in Tuamotu Archipelago of French Polynesia<sup>38</sup>. Similar system had been suggested for the South Sea oyster *P. maxima* in the Australian waters, at least on an experimental basis. There is increasing realisation that these sedentary molluscs are best farmed in their natural habitat. Developments in ocean engineering technology and management of stocks would decide the future of on-bottom culture.

An aspect of pearl oyster farm management which remains elusive is the control of boring and biofouling organisms, parti-

cularly along the tropical coasts. Albeit on a small scale as compared to shipping, the ubiquitous barnacles can cause severe damage to the pearl culture farm and its stock<sup>7</sup>. Bryozoans, ascidians, seaweeds, bivalve spat etc. are common seasonal foulers on the cages and oysters and can cause biological and economic loss. Shallow coastal farming seems to attract more of these organisms than deep water farming. Boring organisms, particularly the sponges and polychaetes, have been considered a real menace to farms<sup>7,13,22,28</sup>. Periodic cleaning of cages and oysters<sup>3</sup> and treatment with freshwater and dilute formaldehyde solution<sup>29</sup> have been suggested to control the problem.

### RAISING OF PEARL OYSTER STOCK

For many years the pearl culturists of Japan depended on the women divers (*ama san*) for the collection and supply of pearl oysters. As the industry expanded, the demands could not be met. Natural calamities such as earthquake and cold current in Ago Bay resulted in the dwindling of natural stocks during the 1940s<sup>3,12</sup>. This led to the evolution of spat collection technique to catch the young oysters on cultches in the subsurface waters which proved a tremendous success<sup>17,23,33</sup>. For nearly three decades the stock requirements of pearl culture farms were fully met by spat collection on cedar sprigs suspended from rafts floating in the bays. At its peak in 1966 when cultured pearl production in Japan reached an all-time high of 127,460 kg<sup>23</sup>, the industry had used about 11,000 tons of oysters, all raised by spat collection<sup>15</sup>.

The peak was followed by a steady decline in every aspect of pearl culture including the resource. The indiscriminate use of the pearl oyster resource including the practice of shifting culture moving the rafts from one area to another led to deterioration of the quality of the oyster. The problems of pollution in the bays has added another dimension to the degradation of stocks.

Attention then was focussed on hatchery production of pearl oyster. The hatchery technology for production of molluscs was readily available and was adopted for pearl oyster. Pearl oyster hatcheries have become common now and, as reported by Ward<sup>38</sup>, every farmer grows spat in tanks and puts them out in bays in cages after 60 days. The major source of supply is from these hatcheries, supplemented by spat collected from the bays.

Pearl culture industry of Australia, Papua New Guinea, Philippines, Thailand and Burma, is facing shortage of *P. maxima* oysters as the supplies come only from the natural source. Spat collection technique has not so far been successful for

the species. Only recently, larval rearing of *P. maxima* has been accomplished experimentally<sup>27</sup> and culture duration from spat to nucleus implantation size could be long.

Dongonab Bay of Sudan, in the Red Sea, has been a traditional ground for *P. margaritifera* and FAO<sup>14</sup> had developed techniques for raising of the population by spat collection. Revived interest in French Polynesia on this species is opening up new areas in production technology. AQUACOP<sup>11</sup> reported on the limited resources, overfishing problem and the success in spat collection on polyethylene sheets. The species has been recently bred successfully in India experimentally (unpublished). In Papua New Guinea, both blacklip (*P. margaritifera*) and goldlip (*P. maxima*) oysters have been bred and spat collection of blacklip oysters on plain nylon rope has been extremely successful<sup>18</sup>.

#### RECENT EXPANSION OF INDUSTRY IN REGIONS OTHER THAN JAPAN

Since 1966, Japanese production of cultured pearls has been on the decline. From 127 tons of marine pearls in 1966 it had dropped to 34 tons in 1973<sup>23</sup>. This has been due a slump in the pearl market caused by the dumping of poor quality pearls produced through short culture. Mizumoto<sup>23</sup> predicted that the maximum pearl production in the future was expected to be about 35 tons/year of *Pinctada* pearl and about 10 tons/year of freshwater pearl. In the more recent years, while marine production has slightly exceeded this projection, freshwater production is still in the order of 5 tons.

The early starters of pearl culture such as Australia, Philippines, Thailand, Malaysia, Indonesia, Burma and Papua New Guinea immediately after the post-war years have not improved production due to several problems. Short supply of *P. maxima* which is the mainstay of pearl culture in the Indo-Australian Archipelago region is the major cause which is crippling the industry. The Australian industry has suffered due to an outbreak of an unidentified disease problem in the oyster stocks recently. Sudan which started a project with Japanese collaboration based on *P. margaritifera* had abandoned it due to a mass mortality whose cause could not be determined.

China which started the pearl Buddha technology in the 12th century has recently entered pearl culture, marine as well as freshwater, in a big way in the recent years. Many pearl farms, *Pinctada fucata*-based, are run by the State and Collectives in Guangdong and Guangxi provinces<sup>39</sup>. Besides, artificial spat rearing and pearl production studies have been made on *P. maxima* in Hainan island. It is in the realm of freshwater pearl production, which started in 1970, that China leads the world today with



a production range of 50-80 tons/year, as against about 5 tons/year of Japan<sup>38</sup>.

The United States of America, which has hitherto played only a supportive role to the Japanese pearl culture industry through supplies of shells of freshwater mussels from Tennessee and Mississippi Rivers for production of shell beads as nuclei of cultured pearls, is taking a direct interest in production of cultured pearls<sup>38</sup>. The species of Unionidae (genera *Tritogonia*, *Quadrula*, *Pleurobema*, *Amblema* and *Megalonais*) had been earlier exploited for their natural pearls<sup>3</sup>. There were over 300 U.S. natural pearl dealers in 1920s but there is none now<sup>38</sup>. Freshwater cultured pearl production which has already been started is scheduled to come to the market by 1987. With 24 species of mussels of great colour, variety and vast clean waters, the U.S.A. appears to have a high potential for freshwater pearl culture.

Among the developing countries, Bangladesh has taken freshwater pearl culture seriously. In 1964 natural pearl production from 98 mussel collection centres was 165 kg worth about Tk 1.4 million<sup>2</sup>. *Parreysia corrugata* and *Lamellidens marginalis* are the two main species of mussels in the extensive system of rivers, canals, ponds and lakes.

#### DEVELOPMENTS IN INDIA

The present phase of R & D effort in pearl culture in India which commenced in 1972 has been very successful. The basic technology for *Pinctada fucata* was developed indigenously<sup>4,25</sup>. Subsequently, an approach was made on production of shell-beads from the Indian conch with success at experimental level<sup>30</sup>; multiple production of pearls was achieved<sup>5</sup> and surgical equipments for nucleus implantation were produced<sup>6</sup>. More recently, hatchery technology for production of pearl oyster spat has been achieved<sup>10</sup>. On the farming side, the biofouling and boring problems had been investigated<sup>7</sup> and their control measures suggested<sup>29</sup> and ecological studies have been carried out<sup>31</sup>. Several transfer of technology programmes have been conducted which has lead to the maritime States and Union Territories taking up pearl culture. The pearl culture potential of Andaman and Nicobar Islands has been investigated and *P. margaritifera* stands out as a candidate species in these island besides the possibility of *P. maxima*<sup>9</sup>. The above developments have led to the establishment of a commercial venture in pearl culture which is in production.

There has been no effort whatsoever on freshwater pearl culture in the country. With vast resources of rivers, lakes and ponds in the Gangetic plain and further south, freshwater



pearl culture is a distinct possibility if attempts are made in the right direction, taking China and Bangladesh as examples.

## SCIENCE AND TECHNOLOGY FRONTIERS IN PEARL CULTURE

Due to the fact that the pearl culture industry had, until recently, been managed exclusively by the Japanese businessmen and farmers, not only in Japan but also outside, research on pearl culture had also been confined to Japan. The National Pearl Research Laboratory of Japan had been the centre of these research activities, besides some universities. With the recent reorganisation of aquaculture research in Japan, pearl culture has become a subject under the National Research Institute of Aquaculture. Australia has carried out need-based research; particularly the recent problem of widespread disease in the *P. maxima* stocks has come under special investigation. With the expansion of pearl culture in countries such as China, U.S.A., India, Bangladesh, French Polynesia and Papua New Guinea, research thrusts on several aspects of pearl culture are also expanding in traditional as well as frontier areas of pearl culture technology which would serve the local needs of the industry as well as enlarge our scientific knowledge.

Improvement of quality of cultured pearls, thereby its value, is receiving the highest priority in Japan. Having faced the pearl crash after 1966 caused by over production and poor quality, the Japanese scientists are very conscious on this aspect. Application of genetics has received due recognition. It has been confirmed by selective breeding experiments that pearls without yellow pigments can be produced more effectively<sup>35-37</sup>. Results of the genetic experiments would lead to improvement of pearl oyster stocks through hatchery production, resulting in stocks yielding pearls of desired quality, resistant to microbial diseases, adaptable to environmental stress, having high growth potential and other desirable characteristics.

A good deal of research has been carried out on mineralisation and spectral characteristics of pearls to understand the formation of organic matrix and crystalline microlayers, ultrastructure of nacre and the causes of colour, lustre and iridescence of pearl<sup>32,34</sup>. The nature and function of extrapallial fluid secreted by the outer epithelium of the mantle, the root cause of pearl formation, is under detailed investigation. These studies are bound to improve the quality of pearls through application of biotechnology.

Tissue culture of pearl oyster mantle has been pursued for many years with the object of isolating and culturing the cells responsible for the secretion of fine aragonite crystals of calcium carbonate which gives the gem quality to the pearl.

*In vitro* culture of mantle epithelium of *P. fucata* resulted in sheet-like accumulations of a large number of migrated cells derived from the explant consisting of roundish epithelial cells, pigmented epithelial cells, spindle-shaped muscle cells or string-like muscle cells and deposition of organic substances has clearly been seen<sup>20</sup>. Colonies derived from epithelial-like cells have been established for *P. fucata* and *Haliotis discus*<sup>21</sup>. It has been recently reported that the following process has been developed: a fraction of cell suspending liquid, arising out of mantle epithelial tissue culture, is injected around the nucleus implanted in the gonad of the pearl oyster, and the cells in suspension would form the pearl sac (*Technocrat*, 18(4), 1985). Success on these lines will open up possibilities of controlling the quality of pearls.

With regard to species, there appears to be scope for improving the techniques for *P. margaritifera* for production of high quality black pearls of sizes larger than what is produced today. French Polynesian efforts in this direction would be rewarding<sup>11,38</sup>. Freshwater mussel *Hyriopsis schlegelii* is amenable to produce nucleated round pearls of large size and high value and the techniques to be improved for achieving this would need greater understanding of the animal's biology and physiology.

The pearl oyster resources need greater attention than has been paid hitherto in view of the environmental deterioration in several areas of pearl culture. In Japan, *P. fucata* wild stocks from different regions have been mixed up indiscriminately to a level that the quality of oyster, in terms of production of pearls of good quality, has degenerated. Attention has to be paid on genetically characterising the wild stocks in their different regions of occurrence and preserving such stocks for judicious use in pearl culture. Pearl oyster diseases have been very little understood and there is greater need to initiate and intensify research in this area, considering the problems faced in *P. maxima* stocks of Australia in this decade and the mass mortality of *P. margaritifera* in Sudan during the last decade.

Hatcheries for raising pearl oyster resource for culture have come to stay in Japan as a major source of supply. Larval rearing procedures for temperate and tropical species of bivalves have been developed<sup>10,19</sup>. However, there seems to be some problems in large scale production of spat of *P. margaritifera*<sup>11</sup> and *P. maxima*<sup>27</sup> which would require attention. In breeding programmes in the hatcheries, the genetic qualities of stocks will have to be considered against the requirements of pearl culturists.

The farming technology has developed for various species and regions based on the local requirements and resources. Ocean engineering is a relatively new discipline which may examine the problems to make the different methods more efficient

and cost-effective. Some of the regions are prone to cyclones, typhoons, hurricanes, monsoons and high tidal amplitudes and open sea farming in such areas is a high risk venture.

Pearl culture is becoming a hi-tech area in Japan to protect quality and increase value. China appears to go in for low-tech venture with labour orientation and high gross production. Countries such as Australia having shortage of labour opt for higher technology to manage production. The U.S.A. is just entering the field and would go in for advances in technology. The scenario of pearl culture, which is changing as above, appears to be conducive for further S&T inputs in the industry.

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#### REFERENCES

1. Abbott, R.T., 1972. Kingdom of the Seashell. Hamlyn Publishing Group Ltd., London, 256pp.
2. Ahmed, M., 1982. Bangladesh - Country report. In: *Bivalve culture in Asia and the Pacific. Proceedings of a workshop held in Singapore. 16-19 February, 1982.* Edited by F. Brian Davy and M. Graham. International Development Research Centre, Ottawa, 20.
3. Alagaraswami, K., 1970. Pearl culture in Japan and its lessons for India. *Proceedings of Symposium on Mollusca.* 3:975-993. Marine Biological Association of India.
4. Alagaraswami, K., 1974a. Development of cultured pearls in India. *Current Science*, 43:205-207.
5. Alagaraswami, K., 1974b. Results of multiple implantation of nuclei in production of cultured pearls. *Indian Journal of Fisheries*, 21:601-604.
6. Alagaraswami, K. and G.S. Sivarajan, 1975. Surgical equipments for pearl culture. *Indian Journal of Fisheries*, 22:231-235.
7. Alagaraswami, K. and A. Chellam, 1976. On fouling and boring organisms and mortality of pearl oysters in the farm at Veppalodai, Gulf of Mannar. *Indian Journal of Fisheries*, 23:10-22.

8. Alagarwami, K.. 1982. Review on controlled breeding of bivalves of aquaculture importance. In: *Progress in invertebrate reproduction and aquaculture*. Edited by T. Subramaniam and S. Varadarajan, Madras University, Madras, 194-202.
9. Alagarwami, K., 1983. The black-lip pearl oyster resource and pearl culture potential. *CMFRI Bulletin*, 34:72-78.
10. Alagarwami, K., S. Dharmaraj, T.S. Velayudhan, A. Chellam, A.C.C. Victor and A.D. Gandhi, 1983. Larval rearing and production of spat of pearl oyster *Pinctada fucata* (Gould). *Aquaculture*, 34:287-301.
11. AQUACOP, 1982. French Polynesia - Country report. In: *Bivalve culture in Asia and the Pacific. Proceedings of a workshop held in Singapore 16-19 February, 1982*. Edited by F.B. Davy and M. Graham, International Development Research Centre, Ottawa, 31-33.
12. Cahn, A.R. 1949. Pearl culture in Japan. *Fisheries Leaflet*, U.S. Fish and Wildlife Service, 357, 91pp.
13. Dharmaraj, K. and N.B. Nair, 1983. Wood-boring organisms in relation to aquaculture along the coasts of India. In: *Proceedings of the symposium on Coastal Aquaculture. Held at Cochin, from January 12 to 18, 1980. Part 2 : Molluscan Culture*, Published by Marine Biological Association of India, Cochin, 684-699.
14. FAO, 1962. Report to the Government of Sudan on the Sudanese shell industry and Red Sea fisheries, FAO/EPTA Rep. (1489), based on the work of William Reed, 47pp.
15. Furukawa, A. 1973. Present status of Japanese marine aquaculture. In: *Coastal Aquaculture in the Indo-Pacific Region*. Edited by T.V.R. Pillay, Fishing News Books, London, 29-47.
16. Hancock, D.A. 1973. Kuri Bay pearls, some of finest in the world. *Australian Fisheries*, 32(4):11-12.
17. Kafuku, T. and H. Ikenoue (Eds). 1983. Modern methods of aquaculture in Japan. Kodansha Ltd., Japan and Elsevier Scientific Publishing Co., Amsterdam, 216pp.
18. Lock, J.M, 1982. Papua New Guinea - Country report. In: *Bivalve culture in Asia and the Pacific. Proceedings of a workshop held in Singapore, 16-19 February, 1982*. International Development Research Centre, Ottawa, 53-34.
19. Loosanoff, V.L. and H.C. Davis, 1963. Rearing of bivalve mollusks. In: *Advances in Marine Biology*, Edited by

F.S. Russel. Academic press, London 1:1-136.

20. Machii, A. 1974. Organ culture of mantle tissue of the pearl oyster *Pinctada fucata* (Gould). *Bulletin National Pearl Research Laboratory*, 18:2111-2117.
21. Machii, A., K.T. Wada, S.T. Townsley, K. Sasaki and M. Awaji, 1985. Application of invertebrate cells in vitro. A Satellite Symposium of the Third International Cell Culture Congress, 14 September 1985, Sendai, Japan, Abstract p. 14.
22. Mizumoto, S. 1964. Studies on disease of the shells of the pearl oyster (*Pinctada martensii*), 1. On the species of parasitic polychaetes in shells, the condition of the damages and the extirpation technique. *Bulletin National Pearl Research Laboratory*, 9:1143-1155.
23. Mizumoto, S., 1979. Pearl farming in Japan. In: *Advances in Aquaculture*, Edited by T.V.R. Pillay and Wm.A. Dill. Fishing News Books Ltd., Farnham, 381-385.
24. Morse, D.E., H. Duncan, N. Hooker and A. Morse, 1977. An inexpensive chemical method for the control and synchronous induction of spawning and reproduction in molluscan species important as protein-rich food resources. *FAO, Fisheries Bulletin*, 200:291-300.
25. Alagarwami, K. and S.Z. Qasim, 1973. Pearl Culture - Its potential and implications in India. *Indian Journal of Fisheries*, 20:533-550.
26. Shirai, S. 1970. The story of pearls. Japan Publications Incorporation, Japan, 132pp.
27. Tanaka, Y. and M. Kumeta, 1981. Successful artificial breeding of the silver-lip pearl oyster, *Pinctada maxima* (Jameson). *Bulletin of National Research Institute of Aquaculture*, 2:21-28.
28. Thomas, P.A., K.K. Appukuttan, K. Ramadoss and S.G. Vincent. 1983. Calcibioticological investigations. *Marine Fisheries Information Services* (T & E Services). 49:1-13. Central Marine Fisheries Research Institute Cochin.
29. Velayudhan, T.S. 1983. On the occurrence of shell boring polychaetes and sponges on pearl oyster *Pinctada fucata* and control of boring organisms. In: *Proceedings of the Symposium on Coastal Aquaculture*. Held at Cochin, from January 12 to 18, 1980. Part 2: Molluscan culture, Published by Marine Biological Association of India, Cochin, 614-618.
30. Velu, M., K. Alagarwami and S.Z. Qasim, 1973. Technique of producing spherical shell beads as nuclei for cultured

- pearls. *Indian Journal of Fisheries*, 20:672-676.
31. Victor, A.C.C., 1982. Ecological conditions of the pearl culture farm at Veppalodai in the Gulf of Mannar, In: *Proceedings of the symposium on Coastal Aquaculture*, held at Cochin from January 12 to 18, 1980. Part 2: Molluscan culture. Published by Marine Biological Association of India, Cochin, 619-626.
  32. Wada, K. 1972. Nucleation and growth of aragonite crystals in the nacre of some bivalve molluscs. In: *Biomineralization Research Reports* (H.K. Erben, Ed.) F.K. Schat-tauer Verlag, Stuttgart, 141-159.
  33. Wada, K. 1973. Modern and traditional methods of pearl culture. *Underwater Journal*, 5(1):28-33.
  34. Wada, K., 1983. Spectral characteristics of pearls. *Gem News*, 14(4):95-103.
  35. Wada, K.T. 1975. Electrophoretic variants of leucin amino-peptidase of the Japanese pearl oyster *Pinctada fucata* (Gould). *Bulletin National Pearl Research Laboratory*, 19:2152-2156.
  36. Wada, K.T., 1984. Breeding study of the pearl oyster, *Pinctada fucata*. *Bulletin of National Research Institute of Aquaculture*, 6:79-157.
  37. Wada, K.T., 1986. Color and weight of shells in the selected populations of the Japanese pearl oyster *Pinctada fucata martensii*. *Bulletin of the National Research Institute of Aquaculture*, 6:1-6.
  38. Ward, F., 1985. The pearl. *National Geographic*, 168(2): 193-222.
  39. Zhong-Qing No. 1982. China - Country Report In: *Bivalve culture in Asia and the Pacific*. Proceedings of a workshop held in singapore, 16-19 Feb. 1982. Edited by F.B. Davy and M. Graham, International Development Research Centre, Ottawa, 21-28.